REMARKS

Claim 12 has been canceled without prejudice; the subject matter of claim 12 has been included in claim 9. Claims 8, 9, 18, 20, 21, 28, and 40 are amended. The amended claims are fully supported by the original specification. For example, support for a nuclear localization signal may be found in the paragraph bridging pages 22 and 23. No new matter has been introduced. The amendments are made solely to expedite prosecution of the application, and Applicants reserve the right to prosecute claims of similar or differing scope in subsequent applications.

Applicants respectfully request reconsideration in view of the following remarks. Issues raised by the Examiner will be addressed below in the order they appear in the prior Office Action.

Election/Restriction

The Examiner has acknowledged Applicants' election, with traverse, of Group IV (claims 21, 28, 40, and 43) in the Response filed on April 4, 2005.

Applicants note that the Examiner has examined claims 1-13, 18, 20, and 98-123 with the claims of elected Group IV.

Objection to the Specification

The Examiner has rejected the specification for the recitation of "in red" in a Figure legend referring to a black and white figure. In response, Applicants have amended the specification to remove the recitation of "in red," and recite "in bold" instead, thereby rendering the objection moot. The amendments to the specification are made solely to clarify the mutated codon. No new matter is being introduced.

Rejection of Claims 1-9, 12-13, 18, and 20 under 35 U.S.C. § 102(a) or (b)

Claims 1-9, 12-13, 18, and 20 are rejected under 35 U.S.C. § 102(a) or (b) as being allegedly anticipated by Bibikova et al. (CA), Chandrasegaran et al. (CE), Kim et al. (CU, CT) or Smith et al. (CL1, CM1). Applicants respectfully traverse these rejections.

The standard for anticipating a claim is clearly outlined in MPEP 2131, and this standard is further supported by the Courts. "A claim is anticipated only if each and every element as set forth in the claim is found, either expressly or inherently described, in a single prior art reference." *Verdegaal Bros. v. Union Oil Co. of California*, 814 F.2d 628, 631, 2 USPQ2d 1051, 1053 (Fed. Cir. 1978).

Applicants have amended independent claims 8, 9, 18, and 20 to clarify that the chimeric nucleases recited in these claims each comprise a nuclear localization signal (NLS). Support for the amendments can be found throughout the specification (e.g., the paragraph bridging pages 22 and 23).

Bibikova et al. (CA, 2001) disclose a method of injecting a chimeric nuclease directly into *Xenopus* oocytes. Chandrasegaran et al. (CE, 1999) provide only a review of the literature relating to chimeric restriction enzymes. Kim et al. (CU, 1998) disclose a specific chimeric restriction endonuclease, GAL4-FokI, for cleaving Gal4 sites in vitro. Kim et al. (CT, 1994) disclose construction of a chimeric nuclease by linking the Drosophila Ultrabithorax homeodomain (Ubx) to the cleavage domain (F_N) of FokI restriction endonuclease. Smith et al. (CL1, 1999) disclose construction of a chimeric restriction enzyme, ΔQNK-F_N, and testing of this chimeric enzyme in vitro. Smith et al. (CM1, 2000) describe the importance of dimerization of the cleavage domain (F_N) in cleaving the target DNA sequences. None of the cited references teach or suggest a chimeric nuclease that comprises a nuclear localization signal, nor do the experimental designs in any of these references suggest that the described chimeric nucleases contained nuclear localization signals. In each instance, the chimeric nucleases were either injected into *Xenopus* oocytes or tested only in cell-free biochemical assays. Therefore, the claims are not anticipated by any of the cited documents.

With respect to claims 8 and 18, the Examiner further states: "[t]he requirement of claims 8 and 18 of recognition sequence within 500 base pairs of the allele for a genetic disorder is all but impossible to determine because it is a very broad term and all such alleles related to genetic disorders have not been determined. It is maintained that this requirement is inherent, absent convincing proof to the contrary" (Office Action, page 3, lines 26-30).

In the first instance, as amended, claims 8 and 18 specify a NLS. This feature is not described expressly or inherently in the prior art, and therefore, the rejection is obviated. Secondly, contrary to the Examiner's assertion, the scope of these claims is clear and readily understood by one of ordinary skill in the art. Applicants have amended independent claims 8 and 18 to clarify that "the DNA binding domain binds to a recognition sequence that occurs at a position in a mammalian genome within 500 base pairs of an allele that is known to contribute to a genetic disorder". A printout from Online Mendelian Inheritance in Man (OMIM) (attached as **Exhibit A)** shows statistics relating to human genetic diversity as of August 16, 2005. 398 genes are indicated as having a known sequence and a phenotypic effect in humans. Therefore, in view of the teachings of the present application, one of ordinary skill in the art can easily use well-known databases such as OMIM and/or publicly available genome sequence information to identify any gene known to participate in a genetic disease, and design nucleases accordingly.

Applicants respectfully submit that none of the cited references meet the limitations of the present claims and thus fail to anticipate the claimed subject matter. Reconsideration and withdrawal of this rejection are respectfully requested.

Rejection of Claims 1-13, 18, 20-21, 28, 40, 43, and 98-123 under 35 USC § 103(a)

Claims 1-13, 18, 20-21, 28, 40, 43, and 98-123 are rejected under 35 USC § 103(a) as allegedly being unpatentable over Bibikova et al. (CA). In particular, the Examiner states that "it would have been obvious to one of ordinary skill in the art to produce all of the elements of the instant claims either in view of the instant reference [Bibikova et al.] or also in view of widely known and used procedures such as insertion of DNA into a vector and/or into a cell . . . [t]he chimeric enzyme contains a nuclear localization signal or it would have been obvious to add it." (Office Action, page 4, lines 18-21).

In making the rejection, the Examiner appears to admit that Bibikova et al. (the single cited reference) fails to describe a chimeric nuclease which comprises a nuclear localization signal, a nucleic acid or vector encoding such a chimeric nuclease, a mammalian cell which comprises such a chimeric nuclease, or any method or use involving such a chimeric nuclease, yet nonetheless asserts that the claimed subject matter is somehow obvious over Bibikova's chimeric nuclease in light of the art.

Applicants respectfully traverse this rejection.

With respect to the methods recited in claim 43, and claims depending from claim 43, Bibikova et al. absolutely fail to disclose or suggest a method in which a repair substrate (as recited in claim 43) is used to change a target sequence in genomic DNA of any cell, let alone a mammalian cell.

Bibikova et al. worked entirely with *Xenopus oocytes*, not mammalian cells, and the substrate DNA to be cleaved by the chimeric nuclease was an artificial, circular plasmid, not genomic DNA. Cleavage of this artificial, non-genomic substrate resulted in intra-episomal recombination, not in the change of a genomic target sequence (see, e.g., Figure 1B). Moreover, Bibikova et al. used chimeric nucleases lacking a nuclear localization signal and introduced these nucleases by direct microinjection. In fact, Bibikova et al. admit that "[s]everal additional issues remain to be addressed to confirm the utility of chimeric nucleases as tools for gene targeting . . . testing the cleavage of **genuine chromosomal targets**" (see, page 296, right column, lines 17-33). Further, Bibikova et al. mention that *Xenopus laevis* oocytes were chosen for these experiments because "these enormous cells have a large capacity for homologous recombination that is readily accessed by microinjection of appropriate substrates . . ." (see page 290, the paragraph bridging the left column and the right column). Accordingly, the results of Bibikova et al., derived entirely from experiments in *Xenopus laevis* oocytes, are by the author's own admission not capable of extrapolation to mammalian cells. Therefore, the teachings of Bibikova neither suggest the claimed methods, nor do they provide one of ordinary skill in the art any reasonable expectation of success in performing targeted modification of genomic DNA in mammalian cells.

With respect to the claimed chimeric nucleases, claims 8, 9, 18, 20, 21, 28, and 40 recite that the chimeric nucleases each comprise a nuclear localization signal. By contrast, Bibikova et al. fail to disclose or suggest a nuclear localization signal. Indeed, in failing to teach or suggest any method of DNA delivery other than microinjection, Bibikova et al. effectively suggest that a nuclear localization signal is dispensable. Moreover, in the absence of any evidence that targeted gene modification can be performed in mammalian cells, one of ordinary skill in the art would have had no motivation to affix a NLS to a chimeric nuclease, and certainly would have

had no reasonable expectation that such nuclease could be used successfully in methods such as those claimed in the present application.

For the aforementioned reasons, Applicants respectfully submit that the outstanding rejection of claims 1-13, 18, 20-21, 28, 40 is improper in that: (a) there is no suggestion or motivation in either Bibikova or the art to modify Bibikova's chimeric nuclease to arrive at the instantly claimed subject matter; and (b) there is no reasonable expectation that would have led one skilled in the art to believe that such a combination would have resulted in chimeric nucleases that are effective for use in vivo or in mammalian cells.

For the above reasons, Applicants respectfully request reconsideration and withdrawal of this rejection.

CONCLUSION

The Examiner may address any questions raised by this submission to the undersigned at 617-951-7000. Should an extension of time be required, Applicants hereby petition for same and request that the extension fee and any other fee required for timely consideration of this submission be charged to Deposit Account No. 18-1945, under Order No. CTCH-P01-016 from which the undersigned is authorized to draw.

Dated: August 19, 2005

Respectfully submitted,

By 

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Attorneys/Agents For Applicant

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Coriell
The Jackson
Laboratory
Human Gene
Nomenclature

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Resources
Genes and Disease
LocusLink
Map Viewer
Sequencing Progress

- Enter one or more search terms.
- Use **Limits** to restrict your search by search field, chromosome, and other criteria.
- Use **Index** to browse terms found in OMIM records.
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OMIM™ - Online Mendelian Inheritance in Man™

Welcome to OMIM, Online Mendelian Inheritance in Man. This database is a catalog of human genes and genetic disorders authored and edited by Dr. Victor A. McKusick and his colleagues at Johns Hopkins and elsewhere, and developed for the World Wide Web by NCBI, the National Center for Biotechnology Information. The database contains textual information and references. It also contains copious links to MEDLINE and sequence records in the Entrez system, and links to additional related resources at NCBI and elsewhere.

You can do a search by entering one or more terms in the text box above. Advanced search options are accessible via the Limits, Preview/Index, History, and Clipboard options in the grey bar beneath the text box. The OMIM help document provides additional information and examples of basic and advanced searches.

The links to the left provide further technical information, searching options, frequently asked questions (FAQ), and information on allied resources. To return to this page, click on the OMIM link in the black header bar or on the graphic at the top of any OMIM page.

NOTE: OMIM is intended for use primarily by physicians and other professionals concerned with genetic disorders, by genetics researchers, and by advanced students in science and medicine. While the OMIM database is open to the public, users seeking information about a personal medical or genetic condition are urged to consult with a qualified physician for diagnosis and for answers to personal questions.

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Restrictions on Use

Allied Resources
Genetic Alliance
Databases
HGMD
Locus-Specific
Model Organisms
MitoMap
Phenotype Davis
Human/Mouse Homology Maps
Coriell
The Jackson Laboratory
Human Gene Nomenclature

Human Genome Resources
Genes and Disease
LocusLink
Map Viewer
Sequencing Progress

OMIM Statistics for August 16, 2005

Number of Entries

	Autosomal	X-Linked	Y-Linked	Mitochondrial	**Total**
* Gene with known sequence	9751	446	48	37	10282
+ Gene with known sequence and phenotype	365	33	0	0	398
# Phenotype description, molecular basis known	1607	143	2	25	1777
% Mendelian phenotype or locus, molecular basis unknown	1345	133	4	0	1482
Other, mainly phenotypes with suspected mendelian basis	2110	148	2	0	2260
Total	15176	903	56	62	**16199**

Synopsis of the Human Gene Map

Chr.	Loci	Chr.	Loci	Chr.	Loci
1	884	9	331	17	536
2	578	10	316	18	136
3	500	11	589	19	608
4	352	12	485	20	218
5	443	13	173	21	124
6	575	14	279	22	233
7	424	15	268	X	548
8	328	16	355	Y	46
Total number of loci: **9329**					